IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Attorney Docket No. 006420.00003)

In the Application of:	)	
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Arora, et al.	)	
	)	Examiner: Clark, Amy L.
Serial No.: 10/525,992	)	
	)	Group Art Unit: 1655
Filing Date: February 28, 2005	)	
	)	
For: Herbal Extract Comprising A Mixture of Saponins Obtained From Sapindus Trifoliatus for Anticonvulsant Activity	)	

DECLARATION OF SUDERSHAN K. ARORA UNDER 37 C.F.R. 1.132

1. I, Sudershan K. Arora, declare as follows.

2. I am an inventor named in the present application. My present title is President – Drug Development of Ranbaxy Research Laboratory, India. My curriculum vitae is attached as Exhibit 1 hereto.

3. I am submitting this Declaration in response to the Office Action mailed December 13, 2007 in the subject patent application.

4. As explained below, the specification as originally filed enables one of ordinary skill in the art to practice the claimed invention. The proper application of the following factors in the present case demonstrates such enablement, as further discussed below.

5. *Nature of the Invention:* While the nature of the invention may be deemed complex, commensurate with the nature of the invention is the level of ordinary skill in

the art. One of ordinary skill in the art, having the benefit of the application as originally filed as well as the Chikara reference, would certainly be able to practice the claimed invention without undue experimentation. Indeed, all one of ordinary skill in the art need do is substitute the claimed invention for what is taught in Chikara.

6. Table 1 of the specification as originally filed shows the benefit of the claimed invention. The specification further details how a composition comprising an aqueous, alcoholic, or hydroalcoholic extract of the pericarp of the fruit of *Sapindus trifoliatus*, comprising from 0.001 to 1.0 % w/v of hederagenin, and at least one pharmaceutically acceptable additive, is capable of prophylactic treatment of migraines – *see e.g.,* page 11, lines 16-20 and page 17, lines 25-29 of the application as originally filed. Additionally, clinical findings, described below, demonstrate that the present extract, and hence the formulation and composition essentially consisting of the same, is useful for the prophylaxis of migraine attack. The mechanism of action that demonstrates the effect of this composition is explained on page 5, lines 23-28, page 6, lines 21-31, and page 20, lines 11-22 of the application as originally filed. I have been made aware, however, that there is no requirement for patentability to demonstrate a mechanism of action, only that the specification enables one of ordinary skill in the art to practice the claimed invention without undue experimentation. Although the animal test examples did not include alcoholic and hydroalcoholic extracts of the fruit of *Sapindus trifoliatus*, it is not necessary for them to do so because all three types of extracts contained "the principal saponins and other primary metabolites." (Page 16, line 6 through page 17, line 13 of the specification as originally filed). A dried *Sapindus trifoliatus* extract was used in the working examples, so the solvent was not included in the pharmaceutical preparation. Because the extracts contained the same active ingredients, there would not have been a significant difference in the anticonvulsant activity of extracts obtained with alcoholic or hydroalcoholic solutions as opposed to water.

7. *Breadth of the Claims:* The claims are no broader than the enabling specification. The Office Action does not discuss this factor in connection with the present application. Instead, the Office Action appears to discuss this factor in

connection with wholly unrelated application, i.e., one that claims a "transfer factor" wherein the "transfer factor is a mammalian transfer factor, and the at least one support component is bitter melon and Indian kino . . . "

8. *Guidance of the Specification and Existence of Working Examples:* The Office Action recognizes that the specification describes an aqueous extract of *Sapindus trifoliatus* displays binding affinity for $GABA_A$ agonistic site in bovine cerebellum, and in Glutamate AMPA site in rat forebrain, Glutamate Kainate site in rat forebrain, Glutamate NMDA site in rat forebrain, Glutamate NMDA Glycine (strychenine-insensitive site) in rat cortex and hippocampus, GABA chloride TBOB in rat cortex, Glutamate chloride in rat cerebellum, and Sodium site 2 in rat forebrain when in higher concentrations (see page 19, Table 1). The Office Action states that there is no description of how these studies were conducted or how the results were obtained. As explained below, one of ordinary skill in the art having the benefit of the specification as originally filed, would know how to conduct such studies and obtain results from such studies. As explained below, the specification as originally filed enables one of ordinary skill in the art to practice the claimed invention and provide prophylactic treatment of migraine mediated through its anticonvulsant activity. Contrary to the Office Action contention that no working examples are shown with regard to the anticonvulsant pharmaceutical composition for nasal administration, examples are indeed provided that evaluated anticonvulsant activity in the maximal electroshock seizure model following intranasal administration of a lyophilized aqueous extract of *Sapindus trifoliatus*, in the pharmaceutically acceptable additive saline, to male Wistar rats – *see* page 20, line 7 through page 21, line 23 of the specification as originally filed. Similarly, the same solution was used to evaluate anticonvulsant activity in the pentylenetetrazole model – *see* page 22, lines 1-24 of the specification.

(1) Working examples of extracts for migraine prophylaxis: The Examiner has commented that the specification lacks working examples with aqueous, alcoholic and hydroalcoholic extracts of pericarp of Sapindus trifoliatus with regard to its prophylactic use in migraine. While there is no example of alcoholic and hydroalcoholic extracts with respect to prophylactic use in migraine, it is respectfully submitted that pages 14 to 27 of

the specification as originally filed describes the composition as well as the extract and working examples. The preparation of the aqueous extract as well as the alcoholic and aqueous alcoholic extract is provided in examples 1 to 4. Example 6 provides a preparation of nasal spray i.e. the composition for nasal route. At page 18 the *in vitro* binding affinity of the extract of Sapindus trifoliatus (3) (which as described at page 14 is dry powder obtained on lyophilization of aqueous extract of Sapindus trifoliatus) is described. It is mentioned that the said activity is conducted at Novascreen®, USA, for GABA Agonist Site, Glutamate AMPA Site, Glutamate Kainate Site, Glutamate NMDA Agonist site, Glutamate NMDA Glycine (Strychnine Insensitive) Site and Sodium Channel. The basis of determining the binding affinity as done by Novascreen®, USA, has been provided. In the same page the use of alcohol extract and hydro alcoholic is also mentioned. The results are provided in Table 1 at page 19. This result shows the activity of the aqueous extract on the various sites. It is further mentioned at page 19 as to how dose dependent binding affinity for various receptor sites takes place. The *in vivo* studies with the same extract indicates prevention of seizure spread on intranasal administration. The anticonvulsant activity for the extract by administering the same intra nasally is demonstrated on male Wistar Rat at page 20 and 21 of the specification as originally filed. This shows the test compounds ability to inhibit MES induced seizure spread. This demonstrates the anticonvulsive activity of the extract (3). Further disclosure at pages 22 and 23 of the specification as originally filed shows that the anticonvulsive activity of extract (3) demonstrated in MES model by intranasal route is without sedation and does not induce or potentiate convulsion of chemical or electrical origin. That anticonvulsive agents act for prophylactic treatment of migraine is described in the Background of the Invention at pages 5, 6 and 7 of the specification as originally filed. Accordingly, it is evident that the present extract of *Sapindus trifoliatus* would act as prophylactic treatment for migraine. Pages 26 and 27 of the specification as originally filed describes that unit formula for nasal spray containing extract of *Sapindus trifoliatus* (3) is prepared and used against migraine. That the active namely the extract (3) acts by binding the specific site is demonstrated and the composition comprises the same active is also disclosed. Accordingly a person skilled in the art would easily prepare composition comprising the said active which would act by binding to specific receptor

sites and bring about anticonvulsive activity which would be required for prophylactic treatment of migraine. In the present case, since the anticonvulsive activity is already known to have effect on migraine, the applicants have provided description of particular composition with extract of *Sapindus trifoliatus*, which by having specific binding affinity for defined receptor sites brings about anticonvulsant activity which is known to act as prophylactic treatment for migraine.

It is further submitted that the present extract (and hence the formulation/ composition essentially consisting of the same) is useful for the prophylaxis of migraine attack is further supported by the clinical findings, which is noted in this Declaration. The mechanism of action that demonstrates the effect of this composition is explained on page 5, lines 23-28, page 6, lines 21-31, and page 20, lines 11-22 of the specification as originally filed. The Examiner's comment of lack of mechanism being provided is respectfully traversed since the binding affinity through *in vitro* as well as *in vivo* anticonvulsant activity is described in the specification as originally filed. Accordingly, it cannot be said that mechanism of action that demonstrate the claimed composition having the claimed effect is lacking.

(2) Working example with regard to the pharmaceutical composition provided nasally: In the present case, the description clearly describes how to form the extracts and the composition. Also, the various types of extracts other than the aqueous form are also taught in the specification. Following the same, a person ordinary skilled in the art can easily prepare the formulation in accordance with the teachings of the specification as originally filed. The binding affinity of the composition is due to the extracts described in the specification, and the effect of such binding is already demonstrated. The other components of the composition is the additive which those skilled in the art will recognize does not play a role in the binding as claimed. Further, the examples teach preparation of the extract as well as the composition comprising the same.

The enablement requirement is met in the present case since the specific extract is described. Applicants' originally filed disclosure of a working of aqueous extract, and the description of other extracts and their preparation clearly meet the enablement requirement as required by Section 112. It is my understanding that the enablement

requirement is satisfied if, given what one of ordinary skill in the art already knows, the specification teaches those in the art enough that they can make and use the invention without "undue experimentation." It would be expected that the person ordinary skilled in the art would easily be able to use the described extract of the present invention with any of the solvent groups also described in the specification, taking the lead from the representative solvent of each of the groups described in the specification.

In the present case, the working examples of the invention with representative of aqueous extract is described, and the working with the alcoholic and hydroalcoholic extract would be similar and considered routine work and cannot be regarded as "undue experimentation" in view of the teachings of governing case law. The description of working aqueous extract ought to be regarded as enabling for alcoholic and aqueous alcoholic extracts. It is further respectfully submitted that generic disclosure in the document should be considered as enabling for similar moieties. Accordingly, it is respectfully submitted that presence of demonstration of the aqueous extract and its working example fulfills the enablement requirement for alcoholic as well as aqueous alcoholic extracts.

Further, the preparations of the alcoholic and hydroalcoholic extracts are enabled in the specification as originally filed. Examples 2, 3 and 4 of the specification as originally filed (*see* page 278, line 6 through page 29, line 2) describe the extraction of pericarp of *Sapindus trifoliatus* in alcohols like n-butanol, iso-propanol, and aqueous ethanol, respectively.

In the specification preparation of the claimed composition is well described at page 25, line 25 through page 27, line 11. In particular, at page 26, line 21 through page 27, line 6, under the Table III, a typical nasal spray has been well described. In the Example-6 (page 29, lines 19-32 of the original specification), such a nasal spray is enabled through a working example.

The present formulation comprising the active ingredient has been shown to be efficacious during clinical trial in humans. A summary of clinical trial report is given below.

As per general practice followed in drug discovery, scientists carry out *in vitro* receptor binding studies and *in vivo* animal efficacy with the drug substance (test protocols do not favor evaluation of finished dosage forms in preclinical models).

Such a study is enabled in the specification as originally filed (*see in vitro* and *in vivo* descriptions at page 17, line 18 through page 27, line 11 of the originally filed specification), and any person skilled in the art would be able to follow the teachings of the specification without undue experimentation.

Even the receptor binding studies given in Table I, at page 19 of the specification was also carried out using the lyophilized extract [3].

Furthermore, the therapeutic potential of the final formulation consisting essentially of the said extract, in the form of a nasal spray as for an example, was exhibited in clinical studies following the teachings provided in the originally filed specification. The summary of the studies is provided by way of this Declaration.

The preparations of the alcoholic and hyrdoalcoholic extracts are enabled in the specification as originally filed. Examples 2, 3, and 4 of the specification as originally filed (*see* page 28, lines 6 through page 29, line 2) describe the extraction of pericarp of *Sapindus trifoliatus* in alcohols like n-butanol, iso-propanol, and aqueous ethanol, respectively. Following the teachings provided in the specification, extracts of *Sapindus trifoliatus* can be prepared by those of ordinary skill in the art without undue experimentation. For example, extracts of *Sapindus trifoliatus* were prepared in the laboratory in isopropyl alcohol (LL-7571), 50% ethanol (LL-7572) and n-butanol (LL-7573) following the teachings provided in the specification. The chemical composition and *in vitro* receptor binding profiles of these three extracts were compared with that of the aqueous extract. Chromatographic studies show that chemically the 50% aqueous-alcoholic extract is similar to the aqueous extract (*see* the Fig. 1 for TLC and Fig. 2 for HPLC below). The concentration of more polar compounds, however, are less in n-butanol and isopropyly alcohol (IPA) extracts. The binding profile of LL-7572 (i.e., 50% ethanolic extract) shows similarity to the profile exhibited by the aqueous extract of the present invention (*see* "Receptor Table" at page 10 of this Declaration for the receptor binding study at 250 μg/ml). This is consistent with the chromatographic profile mentioned above. Although LL-7571 and LL-7573 have receptor binding profiles not

identical to the aqueous extract, the binding affinity was greater than 50% for at least four of the eight binding sites (Table A, receptor binding study).

Fig. 1 : Thin layer chromatography of aqueous, alcoholic and hydroalcoholic extracts of the pericarp of *Sapindus trifoliatus*.

Spot no. (L to R)	Extract Taken	Sample ID
1	Aqueous Ext.	SKJ/28/33-1
2	IPA extract (LL-7571)	SKJ/28/33-2
3	50% Ethanolic Extract (LL-7572)	SKJ/28/33-3
4	n-BuOH Extract (LL-7573)	SKJ/28/33-4

TLC Condition

Concentration of sample : 15 mg/ml [MeOH : Water, 75:25]
TLC Plate : Silica gel 60 F254 [Merck]
Volume applied : 5µl
Solvent System (mobile phase) : Ethyl acetate: Methanol: Water: Glacial acetic acid

[8.5: 1: 0.5: 0.05]

Spray reagent : Vanillin Sulphuric acid
Visualization : After heating at 110° C

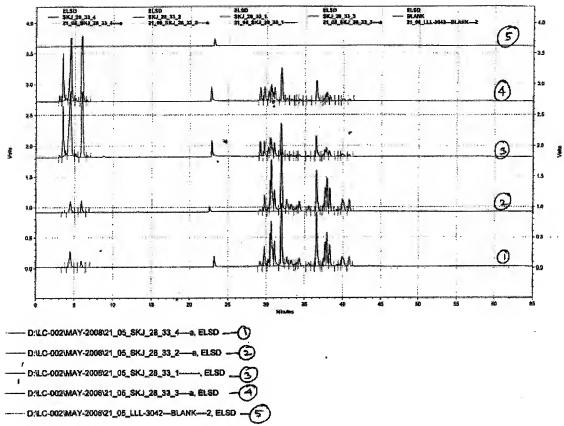


Fig. 2 : High performance liquid chromatography of aqueous, alcoholic and hydroalcoholic extracts of the pericarp of *Sapindus trifoliatus*.

Chromato-gram No.	Extract taken	Sample ID
1	n-Butanol extract (LL-7573)	SKJ-28-33-4
2	IPA extract (LL-7571)	SKJ-28-33-2
3	Aqueous extract	SKJ-28-33-1
4	50 % Ethanolic extract (LL-7572)	SKJ-28-33-3

HPLC conditions:

Concentration of the sample : 2.4 mg/ml in water
Column : Purosphere STAR, RP-18, 5µ, 250 x 4.6 mm (MERCK)
Buffer : 0.1 % formic acid
Mobile phase : Buffer/Acetonitrile (Gradient system)
Flow : 1ml/min
Injection volume : 20 µl
Detector : ELSD (Evaporation temp; 50°C ; Gain 8)

Receptor Table: Receptor binding study at 250 µg/ml:

Receptor	% Inhibition			
	Aq. Extract of *S. trifoliatus* (*ref.* Table I, page 6)	**LL 7571**	**LL 7572**	**LL 7573**
GABA A, Agonist Site	**102.40**	**95.74**	**99.35**	**96.04**
Glutamate, AMPA Site	87.36	-9.45	46.60	-4.89
Glutamate, kainate Site	87.29	23.52	41.59	15.12
Glutamate, NMDA agonist Site	**98.14**	**50.80**	**82.16**	**55.73**
Glutamate, NMDA glycine (Strychnine insensitive) site	85.33	12.92	**58.97**	44.13
GABA chloride,TBOB	**85.03**	**101.18**	**91.35**	**93.23**
Glutamate chloride	89.49	44.48	23.28	21.29
Sodium site 2	**69.54**	**96.07**	**76.20**	**96.35**

** NOVASCREEN® (Caliper Life Sciences), USA, at which selected receptor binding affinity studies described in the specification (see page 18, lines 5-8) were carried out, uses a criteria of 50% inhibition or greater to qualify a compound as active in binding experiments*

The Office Action recognizes that the specification describes an aqueous extract of *Sapindus trifoliatus* displays binding affinity for $GABA_A$ agonistic site in bovine cerebellum, and in Glutamate AMPA site in rat forebrain, Glutamate Kainate site in rat forebrain, Glutamate NMDA site in rat forebrain, Glutamate NMDA Glycine (strychenine-insensitive site) in rat cortex and hippocampus, GABA chloride TBOB in rat cortex, Glutamate chloride in rat cerebellum, and Sodium site 2 in rat forebrain when in higher concentrations (see page 19, Table 1). The Office Action states that there is no description of how these studies were conducted or how the results were obtained. As explained below, there are standard test protocols that a person of ordinary skill in the art would know about and would understand how to carry out in order to obtain and interpret results from the studies described in the specification as originally filed. Receptor binding studies of the extract of *Sapindus trifoliatus* demonstrated the ability to inhibit the binding of a selective radiolabeled ligand to the respective binding site and is measured as bound/unbound amount of radioactivity. For example, test substances are routinely evaluated for any binding towards GABA-A agonist site using its specific ligand, GABA, which is radioactively labeled with ^3H for the purpose of detection. This ligand binds to GABA-A agonist site with a particular affinity (in terms of the amount of radioactivity associated with the receptor preparation). In the presence of the *Sapindus trifoliatus* extract as demonstrated in the present invention, this binding may be either

unaffected or reduced depending the relative affinities of the pharmaceutical preparation to the binding site in question. This study has been well described by Novascreen®, USA, under the *in vitro* studies at pages 17, line 18, through page 19, line 9, of the specification as originally filed, and the results are tabulated in Table I on page 19 of the originally filed specification. In that study, it was found that the extract of the present invention at a concentration 2.5µg/ml, reduced the binding of radioactively labeled GABA (^{3}H-GABA) to $GABA_A$ agonist site by 50.92%, which means that the said extract shares some affinity to $GABA_A$ receptor. At the same concentration (2.5µg/ml) the said extract did not significantly affect the binding of other radiolabeled ligands to their respective binding sites, indicating a greater affinity of the extract of the present invention to GABA-A receptor, as compared to other receptors. As previously noted, Novascreen®/Caliper Life Sciences, USA uses a criterion of 50% inhibition or greater to qualify a compound as active in binding experiments. A set of assay protocols for the respective receptors obtained from Caliper Life Sciences is attached as Exhibit 6. *See also* the website of Caliper Life Sciences (http://www.caliperls.com/products/contract-research/in-vitro/ion-channels/).

As explained below, the specification as originally filed enables one of ordinary skill in the art to practice the claimed invention and provide prophylactic treatment of migraine mediated through its anticonvulsant activity. Contrary to the Office Action contention that no working examples are shown with regard to the anticonvulsant pharmaceutical composition for nasal administration, examples are indeed provided that evaluated anticonvulsant activity in the maximal electroshock seizure model following intranasal administration of a lyophilized aqueous extract of *Sapindus trifoliatus*, in the pharmaceutically acceptable additive saline, to male Wistar rats – *see* page 20, line 7 through page 21, line 23 of the specification as originally filed. Similarly, the same solution was used to evaluate anticonvulsant activity in the pentylenetetrazole model – *see* page 22, lines 1-24 of the specification. Additionally, preparation for the claimed composition is well described from page 25, line 25, through page 27, line 11. In particular, on page 27 under Table III, a typical nasal spray has been elaborately described. In the Example 6, such a nasal spray is enabled through working example. The formulation comprising the active ingredient has been shown to be efficacious during

clinical trial in humans, which is given below. Moreover, as per general practice in drug discovery by one skilled in the art, *in vitro* receptor binding studies and *in vivo* animal efficacy studies are carried out with the drug substance. Such a study is enabled in the specification (*see in vitro* and *in vivo* sections from page 17, line 18, through page 27, line 11, and a person of ordinary skill in the art would be able to follow the teachings of the specification without undue experimentation. Further, the receptor binding studies given in Table I, page 19 of the specification were carried out using the lyophilized extract [3]. Furthermore, in terms of determining which binding sights are responsible for the different aspects of seizure, as described on page 5, line 30, through page 6, line 19, herbal extracts act through multiple mechanisms. However, the therapeutic potential of the said extract in, for example, the form of a nasal spray, is exhibited in the clinical studies as described below.

Summary of Clinical Trials conducted with the composition/formulation of the present invention:

A proof of concept study was conducted to evaluate the tolerability profile of 0.15%, 0.25%, 0.5%, 1% and 3% of nasal formulation of LLL-2011 (LLL-2011 is the extract [3] as disclosed in the specification as originally filed). The botanical drug product LLL-2011 was delivered as a nasal spray in Phase II clinical trials containing lyophilized aqueous extract of *S. trifoliatus.* Ten healthy subjects were randomized to each group. Drug concentrations of 0.15% to 1% were found to produce mild to moderate irritation of mucus membrane. Drug concentration of 3%, however, was found to produce severe irritation. Thus, the lowest dose and the highest dose in the tolerated dose range i.e., 0.15% and 1% nasal formulation of LLL-2011 were considered for further evaluation in phase I/II clinical trial to establish safety and efficacy. Since the formulations contain extract of *Sapindus trifoliatus* that is commonly used in various preparations, the Phase I study for tolerability profile and Phase II study for efficacy evaluation were reviewed together in migraine patients.

Primary objectives of the study: To determine optimal dosage of LLL-2011 in reducing the frequency, intensity, duration and total pain index with two different doses of LLL-2011 as compared to placebo in the preventive treatment of common migraine.

Secondary objectives of the study: To determine local as well as systemic tolerability of LLL-2011 at different dosage regimens of LLL-2011, and to determine frequency of requirement for rescue medication during the active treatment period.

Phase I/II clinical trial:

Data obtained from the studies conducted at reputed medical research centers in India, e.g., Post Graduate Institute of Medical Education and Research at Chandigarh, Sterling Hospital at Ahmedabad and Deenanath Mangeshkar Hospital at Pune are given below. The study design included placebo-control, randomized, double blind, and parallel group methodology. The study enrolled 151 patients of which 84 patients who completed the study were found to be suitable for statistical analysis. The patients were randomized to treatment groups consisting of 0.15% or 1% nasal formulation of LLL-2011 or placebo.

Results:

Results of the study demonstrate that LLL-2011 intranasal spray in the doses of 0.15% and 1% are effective in the prophylactic treatment of migraine. Significant reduction in migraine attacks from baseline was observed in LLL-2011 (0.15%) and LLL-2011 (1%) treatment groups. However, between groups statistical significance was not detected. Clinically, both LLL-2011 (0.15%) and LLL-2011 (1%) treatment groups showed more than 50% response rate in comparison to placebo response, which was <50%. The LLL-2011 (1%) formulation produced greater effect in reducing migraine attacks than the LLL-2011 (0.15%) formulation. Only 3 out of 42 patients on LLL-2011 (1%) treatment withdrew from the study due to local intolerance i.e., severe nasal burning sensation. Other adverse events reported were sneezing and itching. Otherwise both active formulations were moderately well tolerated.

The specification as originally filed enables one of ordinary skill in the art to practice the claimed invention and provide prophylactic treatment of migraines mediated through its anticonvulsant activity.

Predictability and State of the Art: The specification as originally filed enables one of ordinary skill in the art to practice the claimed invention without undue experimentation and provide prophylactic treatment of migraines mediated through its anticonvulsant activity.

In Table I on page 19 of the specification as originally filed, eight binding sites were identified as of proposed antimigraine activity of *Sapindus trifoliatus*. Here, the binding data is functionally correlated with *in vivo* studies (MES model). In the specification, *Sapindus trifoliatus* is shown to have anticonvulsant activity in *in vivo* rat MES model (*see* page 20, lines 11-22). Based on published literature cited in the corresponding text, the same anticonvulsant activity is proposed to be due to binding of *Sapindus trifoliatus* towards Glutamate NMDA, Glutamate Kainate, Glutamate AMPA, Glutamate NMDA Glycine and Sodium site 2, as mentioned from lines 8-9 and 11-22 on page 20 of the specification as originally filed. However, the relevance and involvement of other binding sites, i.e., GABA A agonist site, GABA, Chloride, TBOB & Glutamate chloride, is available in various published literature as mentioned on page 5, line 23, through page 6, line 31. In addition, one of ordinary skill in the art would know how to perform human studies without undue experimentation as illustrated in the human studies were performed and reported in paragraph 11 above using the extract [3] as disclosed in the specification as originally filed following the teachings of the present application. It is respectfully submitted that it is not necessary to extrapolate the effects of *Sapindus trifoliatus* extracts on the prophylactic treatment of migraine only from animal studies.

Epilepsy and migraines "share several clinical features and in many instances, respond to the same pharmacological agent." (*See* page 5, lines 17-18 of the specification as originally filed). Thus, drugs known for the treatment or prevention of epilepsy, such as anticonvulsants, would also have a reasonable expectation of success for treatment or prevention of migraines. For example, as explained on page 5, line 30 through page 6, line 19 of the specification, anticonvulsant drugs such as sodium valproate and gabapentine have been demonstrated to be effective at preventing migraines by modulating GABA neurotransmission, and topiramate has been under investigation as a prophylactic agent for migraines as a result of its interaction with AMPA/Kainate glutamate receptors and GABA-A receptors. The extract of *Sapindus trifoliatus* was

shown in Table 1 to have effective binding affinities for "receptor sites viz. GABA-A agonist site, Glutamate-AMPA site, Glutamate-Kainate site, Glutamate-NMDA agonistic site, Glutamate-NMDA glycine (strychnine insensitive) site and Sodium channel (site 2), which are known to have major mediatory role in anticonvulsant activity." (Page 9, line 28 through page 10, line 2). One of ordinary skill in the art would have a reasonable expectation that the *Sapindus trifoliatus* extract could act as a prophylactic agent for migraines because migraines and epilepsy often respond to the same drugs and the *Sapindus trifoliatus* extract has high binding affinities for receptor sites that mediate anticonvulsant activity.

The animal studies conducted and described in the specification, as originally filed, provides reasonable prediction of success of the effect of the claimed anti-migraine medication in humans, and provides basis for why the claim-designated compositions may be useful for providing such an effect.

Amount of Experimentation Necessary: The quantity of experimentation necessary to carry out the claimed invention is low, and the skilled artisan could rely on the instant specification in combination with prior art on how to make and use the claimed pharmaceutical composition for nasal administration.

As to the guidance of the specification and existence of working examples, the Examiner has mentioned that there is no description as to how the studies on the binding affinity were conducted or how the results were obtained. It is respectively submitted that at page 18 of the originally filed specification it is mentioned that *in vitro* receptor binding studies reveal the binding affinity of the extract (3), which has mediatory role in anticonvulsant activity. As mentioned above the said affinity studies were carried out at Novascreen and the basis of their study and correlation with binding affinity were provided in the prior response dated September 25, 2007. It is submitted once again that those of ordinary skill in the art, following the teachings of the originally filed specification, would know how to conduct such studies and correlate the specific binding affinities without undue experimentation. The results thus obtained are provided in Table I of the originally filed specification at page 19. Accordingly, it cannot be said that how

the studies were conducted and results obtained were not known to those of ordinary skill in the art.

Preparation of the aqueous, alcoholic and hydroalcoholic extracts of *Sapindus trifoliatus* are described in detail on page 15, line 26, through page 16, line 8, of the specification as originally filed. The preparations of such extracts are exemplified in the Examples 1 through 4, and the preparation of a nasal formulation is enabled in Example-6 from lyophilized aqueous extract [3]. Further, the preparation of identical formulations by substituting the extract [3] with any of alcoholic or hydroalcoholic extracts is within the ability of one of ordinary skill in the art. The preparation of batches of nasal spray containing a *Sapindus trifoliatus* extract is described in detail on page 25, line 21 through page 27, line 6. The administration of the pharmaceutical formulation is disclosed on page 27, lines 9-11. In view of the specificity of the claimed invention and detailed guidance provided by the specification as well as the level and knowledge of one of ordinary skill in the art, the skilled artisan would not have to conduct an undue amount of experimentation to make and/or use the claimed invention.

In view of the specificity of the claimed invention and detailed guidance provided by the specification as well as the level and knowledge of one of ordinary skill in the art, the skilled artisan would not have to conduct an undue amount of experimentation to make and/or use the claimed invention. The rejection under 35 USC 112, first paragraph, for lack of enablement should be withdrawn.

9. *Predictability and State of the Art:* As explained below, the specification as originally filed enables one of ordinary skill in the art to practice the claimed invention without undue experimentation and provide prophylactic treatment of migraine mediated through its anticonvulsant activity. Epilepsy and migraines "share several clinical features and in many instances, respond to the same pharmacological agent." (Page 5, lines 17-18 of the specification as originally filed) Thus, drugs known for the treatment or prevention of epilepsy, such as anticonvulsants, would also have a reasonable expectation of success for treatment or prevention of migraines. For example, as explained on page 5, line 30 through page 6, line 19 of the specification, anticonvulsant drugs such as sodium valproate and gabapentine have been demonstrated to be effective

at preventing migraines by modulating GABA neurotransmission, and topiramate has been under investigation as a prophylactic agent for migraines as a result of its interaction with AMPA/Kainate glutamate receptors and GABA-A receptors. The extract of *Sapindus trifoliatus* was shown in Table 1 to have effective binding affinities for "receptor sites viz. GABA-A agonist site, Glutamate-AMPA site, Glutamate-Kainate site, Glutamate-NMDA agonistic site, Glutamate-NMDA glycine (strychnine insensitive) site and Sodium channel (site 2), which are known to have major mediatory role in anticonvulsant activity." (Page 9, line 28 through page 10, line 2) One of ordinary skill in the art would have a reasonable expectation that the *Sapindus trifoliatus* extract could act as a prophylactic agent for migraines because migraines and epilepsy often respond to the same drugs and the *Sapindus trifoliatus* extract has high binding affinities for receptor sites that mediate anticonvulsant activity.

10. *Amount of Experimentation Necessary:* As explained below, the quantity of experimentation necessary to carry out the claimed invention is low, and the skilled artisan could rely on the instant specification in combination with prior art on how to make and use the claimed pharmaceutical composition for nasal administration. The preparation of batches of nasal spray containing a *Sapindus trifoliatus* extract is described in detail on page 25, line 21 through page 27, line 6. The administration of the pharmaceutical formulation is disclosed on page 27, lines 9-11. In view of the specificity of the claimed invention and detailed guidance provided by the specification as well as the level and knowledge of one of ordinary skill in the art, the skilled artisan would not have to conduct an undue amount of experimentation to make and/or use the claimed invention.

11. The claimed invention is non-obvious in view of Chikara. The Office Action does not properly recognize the evidence that Chikara teaches away from the claimed invention. There would be no reason for one of ordinary skill in the art to deviate from the teaching of Chikara. Thus, there would be no reasonable expectation of success in deviating from the teaching of Chikara.

12. Since Applicants have fully enabled the invention they are claiming, the teachings of Chikara is not the only teachings that provide enablement for Applicants' invention. Therefore, the Chikara rejection does not teach or suggest the invention for which Applicants have enabled.

13. Contrary to the Office Action, Applicants have shown that an aqueous extract of *Sapindus trifoliatus* provides the instantly claimed effects, and therefore Applicants have fully enabled the invention they are claiming.

14. Table 1 below is a redacted excerpt from the specification of the present patent application (page 19).

Table 1: Disclosure in US 10/525,992

S.No.	Receptor	Percent inhibition with *Sapindus trifoliatus*	
		2.5 µg/ml	**250 µg/ml**
1	GABA A, agonist Site	50.92	102.40
2	Glutamate AMPA Site	5.43	87.36
3	Glutamate Kainate Site	-15.70	87.29
4	Glutamate NMDA agonist Site	7.27	98.14
5	Glutamate NMDA glycine (Strychnine insensitive) site	14.50	85.33
6	GABA chloride TBOB	-5.12	85.03
7	Glutamate chloride	-2.72	89.49
8	Sodium site-2	19.98	69.54

15. A criterion of 50% inhibition or greater may be used to qualify a compound as active in binding experiments. *See* Exhibit 5 to this Declaration (literature specification from Novascreen).

16. As shown in Table 1, at 250 µl/ml, *Sapindus trifoliatus* is qualified as active under the Novascreen criterion.

17. *Emblica officinalis* was tested for binding towards the sites listed in the above table. Receptor binding assays were conducted at Novascreen, U.S.A. The results are summarized in Table 2 hereinbelow (See Exhibit 4).

Table 2: Receptor Binding affinity with the extract of *Emblica officinalis*

S.No.	Receptor	Percent Inhibition with *Emblica officinalis*	
		2.5 µg/ml	250 µg/ml
1	**GABA A agonist Site**	**64.09**	**102.22**
2	**Glutamate AMPA Site**	-10.07	**56.20**
3	Glutamate Kainate Site	-1.12	39.30
4	**Glutamate NMDA agonist Site**	14.90	**71.82**
5	Glutamate, NMDA glycine (Strychnine insensitive) site	0.48	28.21
6	GABA chloride TBOB	-1.95	-0.03
7	Glutamate chloride	28.28	49.24
8	Sodium site-2	7.11	3.93

18. As seen, *Emblica officinalis* at 250 µg/ml fails the Novascreen criterion for Glutamate Kainate site, Glutamate, NMDA glycine (Strychnine insensitive) site, GABA chloride, TBOB, Glutamate chloride, and Sodium site-2.

19. An antimigraine formation prepared as per Chikara was also tested for binding towards the foregoing sites (see Exhibit 4). The results are summarized in Table 3.

Table 3: Receptor Binding affinity with the antimigraine formulation mentioned in Chikara et al patent.

S.No.	Receptor	Percent inhibition (Chikara composition)	
		2.5 µg/ml	250 µg/ml
1	GABA A, Agonist Site	19.21	**95.95**
2	Glutamate, AMPA Site	-0.89	41.69
3	Glutamate, kainate Site	-1.16	25.68
4	Glutamate, NMDA agonist Site	15.26	**66.02**
5	Glutamate, NMDA glycine (Strychnine insensitive) site	4.03	42.60
6	GABA chloride,TBOB	-14.60	-3.77
7	Glutamate chloride	1.95	**84.35**
8	Sodium site 2	13.37	3.30

20. Chikara showed markedly reduced binding relative to the inactive composition. Chikara satisfied the 50% inhibition criterion only for three sub-types of receptors, and failed to satisfy the 50% inhibition criterion for Glutamate AMPA site, Glutamate Kainate site, Glutamate NMDA glycine (Strychnine insensitive) site, GABA chloride, TBOB, and Sodium site 2.

21. The saponin content of *Sapindus trifoliatus* and *Emblica officinalis* were evaluated via high performance liquid chromatography, conducted according to the following protocol:

Quantitative Analysis of Raw Material by High Performance Liquid Chromatography (HPLC)

Preparation of the sample: About 1g of the fruit pericarp powder was accurately weighed into a 250ml round bottom flask (RB Flask), 100ml water was added to the same and the total weight of the flask was noted. The content of the flask was then refluxed for 2hrs at 100°C. After cooling the flask was weighed again and water was added to adjust the lost volume to make up the solution to 250ml. From this sample solution, 20ml was transferred to a 100ml RB Flask and 5ml of 50% methanolic-HCl was added to it. The solution was then refluxed at 100 ^{0}C for 2hrs and then evaporated to dryness. The residue was dissolved in 10ml of diluent (A mixture of THF and Methanol in the ratio 30:70 v/v) and carefully transferred to 50ml volume. The samples were analysed and the saponin content were estimated as hederagenin by a quantitative HPLC method similar to that described hereinafter.

22. The following results were observed:

1. Total saponin content of *Sapindus trifoliatus* pericarp was analyzed as hederagenin and was found to be 2.65% w/w.
2. In case of *Emblica officinalis* fruit, hederagenin content was below the detection limit.
3. In the mixture of *Sapindus trifoliatus* and *Emblica officinalis* (1:1) hederagenin content was 1.01% w/w.

23. These results demonstrate that any hederagenin in the mixture of Chikara originated from the extract of *Sapindus trifoliatus* pericarp.

24. For further verification, thin layer chromatographic analysis was performed. The analysis was performed according to the following protocol.

Thin Layer Chromatography (TLC) Materials and methods

A. Sample Preparation for the finger printing by TLC of 1% nasal spray: 1.5 mL of the sample was taken in a 10 mL volumetric flask and the volume was made up to mark with water. The content was carefully transferred to a 50 mL-separating funnel and extracted with 5 mL of n-Butanol 3 times. The organic layer was collected. The combined extract was transferred to a 50 mL round bottom flask and evaporated to dryness. The residue thus obtained was dissolved in about 2 mL methanol

B. Sample Preparation for Raw material: Around 1.0 gm of sample was taken in a 250 mL round bottom flask. 100 mL of water was added and refluxed at 100 ^{0}C for 2 hours. 10mL of extract was carefully transferred to a 50mL-separating funnel and extracted with 5 mL of n-Butanol 3 times. The organic layer was collected. The combined extract was transferred to a 50mL round bottom flask and evaporated to dryness. The residue thus obtained was dissolved in about 2mL methanol.

C. Mobile Phase: Ethyl Acetate : Methanol : Water (80:10:10)

D. Detection: TLC plate is sprayed with 10% acqueous sulfuric acid, charred by heating and observed visually.

Details Of samples spotted on The TLC plate:

Raw Material:

Spot No	Details	Sample ID
1	*Sapindus trifoliatus*	SKJ-20-181-1
7	(1:1) Mix of *Sapindus trifoliatus* and *Emblica officinalis*	SKJ-20-181-5
13	Emblica officinalis	SKJ-20-181-3

Compositions:

Spot No	Details	Sample ID
2	Composition of the present invention (1% w/v)	SKJ-20-181-2
12	Composition of Chikara et al (1% w/v)	SKJ-20-187-6
18	Composition comprising (1%w/v) of *Emblica officinalis*	SKJ-20-187-4

25. The Figure attached as Exhibit 2 shows the qualitative TLC chromatogram. Spots 1, 7, and 13 are of extracts of the raw materials, namely *Sapindus trifoliatus*, and a 1:1 mixture of these two, respectively. The bands corresponding to sapinons are marked in the figure. *Emblica officinalis* does not show any band corresponding to the saponins, whereas *Sapindus trifoliatus* does show a band corresponding to saponins. The Figure is not intended to be a qualitative TLC chromatogram, and the intensities of the bands are not deemed to have relevance.

26. *In vivo* toxicology studies were performed in rats and dogs:

A. Rats: Antimigraine Formulation (3%) according to Chikara et al. patent, *Sapindus trifoliatus* (3%) and Mixture of *Sapindus trifoliatus* and *Emblica officinalis* (3%; 60:40) were studied for nasal irritancy. None of the formulations were found to be irritant to nasal mucosa, turbinate, bronchi and lungs.

B. Dogs: Antimigraine Formulation (1%) according to Chikara et al. patent, *Sapindus trifoliatus* (1%) and Mixture of *Sapindus trifoliatus* and *Emblica officinalis* (1%) were studied for nasal irritancy. None of the formulations were found to be irritant to nasal mucosa, turbinate, bronchi and lungs.

27. Applicants have assayed saponins as hederagenin in the compositions as compared with the compositions of Chikara et al. The details are provided hereinbelow:

HPLC Assay of Saponins As Hederagenin In Anti-Migraine Nasal Preparation

Reagents used

Formic Acid (AR Grade)
Acetonitrile (HPLC Grade)
Water (Milli Q Grade)
Methanol (HPLC Grade)
Tetrahydrofuran (HPLC Grade)
Hydrochloric acid (AR Grade)

Preparation of Diluent

A mixture of THF and Methanol in the ratio 30:70 v/v was used as a diluent

Preparation of Standard Solution: About 50 mg of Hederagenin working standard was weighed and transferred to a 50 mL volumetric flask. 20 mL of diluent was added and sonicated to obtain a clear solution. The volume was made up using the diluent. 5mL of the standard solution was pipetted into a 50 mL volumetric flask and the volume was made up with methanol. The resulting solution was filtered through a 0.45 μ membrane filter. Estimation of Saponins as Hederagenin content in 1% Nasal Preparation of Chikara et. al. and present invention by acid hydrolysis. 15 mL of the samples were taken separately in a 100mL round bottom flask. 5ml of 50% methanolic hydrochloric acid was added and refluxed at 100 ^{0}C for 2 hours. The solutions were cooled and evaporated to dryness. 10 mL of diluent was added to each residue and sonicated for about 2 minutes. The contents were carefully transferred to 50 mL volumetric flasks and the volume was made up with methanol. The solutions were filtered through a 0.45 μm membrane filters. The above samples were analyzed in HPLC. The chromatographic conditions are as given below:

Instrumentation: A high performance liquid chromatograph system with gradient elution capability, autosampler with cooling chamber (Shimadzu class VP series) or equivalent.

Data handling system (Class VP, version 5.032 or equivalent): Analytical column: A stainless steel column 250 cm long, 4.6 mm in diameter filled with Octadecyl silica gel particle of 5 μm in diameter.(Used Kromasil C-18, 5μm (250 mm x 4.6 mm)

Preparation of Buffer: 1.0 mL of formic acid was added to 1000 mL volumetric flask and made up to the volume with water. The solution was filtered through a 0.45 or finer porosity membrane filter and degassed.

Chromatographic Parameters

Mobile Phase	:	25:75 (Buffer : Acetonitrile)
Flow rate	:	1 mL/min
Detector	:	UV at 205nm
Injection Volume	:	50 µL
Run time	:	20 min

Procedure: 50 µL of standard in triplicate and the specified sample solution in duplicate was injected, into the chromatograph. The chromatograms were recorded and the peak area of the main peak from the chromatographic report was measured. The retention time for Hederagenin was about ~ 7.75min.

Calculations

$$\text{Total saponins as Hederagenin (mg/mL)} = \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100}$$

Where

AT = Average area counts of Hederagenin peak in the chromatogram of the sample solution

AS = Average area counts of Hederagenin peak in the chromatogram of the standard solution

DS = Dilution factor of the standard solution in mg/mL

DT = Dilution factor of the sample solution in mL/mL

P = Percent potency of Hederagenin working standard, on as is basis.

Details of Samples:

S. No.	Batch No	No of injections	Decoding	Figure Number
1	SKJ-22-183	3	Hederagenin Working STD (LLL3344)	Figure 1, 2, 3
2	NP-50-197	2	Formulation by Chikara et al Method	Figure 4, 5
3	LLL3344	3	Hederagenin Working STD	Figure 6, 7, 8

	Hederagenin		(SKJ-22-183)	
4	MB-50-193	2	Formulation by Present invention Method	Figure 9, 10

28. The results obtained are tabulated in the following tables as given below (see also Exhibit 3).

TABLE A: Assay of 1% Anti-Migraine Nasal Preparation, Prepared By Chikara et al

SAMPLE NAME	Quantity taken	Makeup volume (mL)	Concentration	Area	Average area	Assay (mg /mL)
STD	51.2 mg	500	**0.1024**	2844979	**2835323**	
STD POTENCY			(mg/mL)	2839659	RSD	
96.88				2821331	0.44%	
% w/w on as is basis						
NP-50-197	15 mL	50	**0.3**	1219681	**1220211**	
(1% Formulation)			(mL/mL)	1220741	RSD	0.1423
					0.06%	

TABLE B: Assay of 1% Anti-Migraine Nasal Preparation, Prepared By Present invention

SAMPLE NAME	Qtantity taken	Makeup volume (mL)	Conc.	Area	Average area	Assay (mg /mL)
STD	47.1 mg	500	**0.0942**	**2697556**	**2695596**	
STD POTENCY				**2695518**	**RSD**	
96.88				**2693713**	**0.07%**	
% w/w on as is basis						
MB-50-193	15 mL	50	0.3	2868579	2873603	
(1% Formulation)				2878626	**RSD**	0.3243
					0.25%	

It is noted that hederagenin in fact is an indirect estimation of the total triterpenoid saponins present in the *Sapindus trifoliatus* extract. Saponins are glycosides of steroids, steroidal alkaloids or triterpenes found in plants. In cases of triterpenoid saponins the

glycoside units are linked to the triterpene moiety (known as aglycone) at its C-3 position (*see* Exhibit 7, Trease and Evans' Pharmacognosy, Fourteenth edn., Page 293).

However, on hydrolysis of saponins, free aglycone is obtained. By measuring the concentration of the aglycone moiety one can arrive at a particular concentration of the total saponins present in an extract.

In a few occasions the aglycone part of the saponin is hederagenin. Therefore, the applicants would like to emphasize here that hederagenin is a natural product known in the art and the present invention is not directed to the same *per se*. To reiterate, in accordance with the present invention, fruit pericarp of the plant *Sapindus trifoliatus* is extracted in water or alcohol or in a mixture of water and alcohol. What is obtained is a specific range of concentration of a mixture of triterpenoid saponins in the extract. The aglycone part in all saponins is hederagenin (*see* compounds 5-10, page 16 –17 of the specification as originally filed).

The extract obtained in the present invention possesses anticonvulsant activity. It is important for any therapeutic activity to deliver a known quantity of the active substance. In this case, by knowing the quantity of sugars and aglycon, since the ratio is the same, the concentration can be measured through the hederagenin obtained by the acid hydrolysis of the saponins. Hence, the saponin quantity in the extract is measured by estimating the hederagenin content (see lines 20-30 of page 16 and lines 1-13 of page 17 of the specification as originally filed). This is further evident from Row No. 2 of Table III at page 27 of the specification, wherein the active ingredient saponin strength is expressed in terms of % w/v hederagenin content. Thus, measurement of hederagenin is an indirect estimation of the triterpenoid saponins present in the extract. It has been observed that the extract obtained by following the process of the present invention typically contains a mixture of triterpenoid saponins that, when expressed in terms of hederagenin, is in the range of 4-8% w/w of hederagenin. The pharmaceutical composition of the present invention derived from the same extract typically contains such saponins that, when expressed in terms of hederagenin in a similar manner, is in the range of "0.001-1% w/v of hederagenin." In view of the foregoing, claim 1 has been amended to claim "**an aqueous, alcoholic, or hydroalcoholic extract of the pericarp of**

the fruit of Sapindus trifoliatus, comprising <u>of total saponins estimated as hederagenin ranging from 0.001 to 1.0 (%w/v), and…..</u>"

The total saponin content was analyzed as hederagenin (*see* page [insert], *supra* under "Preparation of the sample"). This is consistent to the disclosure of the specification as originally filed at page 17, lines 6-13.

The Examiner contends that hederagenin would be inherently present in *Sapindus trifoliatus* and hence there is no invention in using the same. The Examiner contends that the applicants have not shown that aqueous extract of *Sapindus trifoliatus* provides the instantly claimed effects and therefore has not enabled their invention they are claiming and hence it is only the cited art which is enabling. As noted above, the aqueous extract of *Sapindus trifoliatus* provides enabling disclosure as to the binding affinity as well as providing the anticonvulsant activity. Accordingly, the submissions herein should to be accepted. That anticonvulsant activity is related to prophylactic treatment of migraine is already provided in the Background of the Invention as originally filed, and the Examiner's attention is respectfully drawn to the same to correlate the working examples of the invention. In other words, the present invention shows anticonvulsive activity of the composition and demonstrates anti-convulsive activity to be related to prophylactic treatment of migraine. Accordingly, the composition for prophylactic treatment of migraine is claimed.

29. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 of the laws of the United States, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FURTHER DECLARANT SAYETH NOT

Sudershan K. Arora

Executed on: 10th June 2008
Date

EXHIBIT 1

Sudershan K. Arora, Ph.D.

May 2008 – Present:	President – Drug Development Ranbaxy Research Laboratory Gurgaon, India
Apr 2005- Apr 2008 :	President – Novel Drug Discovery & Development Lupin Research Park Pune, India
2004-2005	Global Head R&D Sandoz GmbG (A Novartis Company) Biochemiestr, 10 6250 Kundl Austria
2000 – 2004	President – New Chemical Entity Research Lupin Research Park (Lupin Limited) Pune, India
1997 – 00	Vice President, New Drug Discovery Research Ranbaxy Laboratories Ltd Gurgaon, India
1993 – 97	Sr. Manager – R&D (Process Research) Biogen Inc, Cambridge, MA, USA
1987 – 93	Sr. Director – Drug Discovery & Process Research Greenwich Pharmaceutical, Fort Washington, PA, USA
1982 – 87	Post-Doc University of Illinois at Chicago, USA (Dr. J. Kagan & Dr. D.L. Venton)
1979 – 82	Manager – Process Research & New Molecules Union Carbide, Bhopal, India
1978 – 79	Lecturer, S.D. College, Pathankot, India
1977	Ph.D. Kurukshetra University (Medicinal Chemistry), India
2004	D.Sc Thesis entitled - Animal PK and toxicology

Areas of Expertise:

Drug Discovery and Development, Process Chemistry (API & new molecules) and Phytochemistry.

Major Accomplishments

- Established state-of-art Lupin R&D Centre in Pune.
- Filed 9 IND Applications (3 – USA & 6 – India)
- BPH Compound is in Phase – II clinical trials
- VLA_4 (Asthma) compound is in Phase – II clinical trials
- Amigra – Anti-migraine (Herbal) compound is in Phase-II clinical trials
- Desoris – Anti-psoriasis (Herbal) compound Phase-II clinical trials completed
- Sudoterb – Anti-TB compound has completed Phase-I clinical trials
- Desoside-P – Anti-Psoriasis single compound completed Phase I clinical trial
- Process Development of various Generic Products/New molecules in India & USA
- 45 Publications in Peer reviewed journals
- 24 US patents
- 11 India Patents
- DST-CSIR-Lupin Collaboration (IICT-HYD, NCL-PUNE, IISC-BANGALORE.)
- Active Participant in NMITLI (CSIR) projects.

Awards

- Honorary Professor for Life, Department of Life Sciences, Bundelkhand University, Jhansi, U.P. (February, 2001)
- Professor A.S.R. Anjaneyulu Award, awarded by Indian Chemical Society, Kolkata (December, 2002)
- SS Katiyar Award – conferred by Indian Science Congress (January, 2005)

Task Force Member/Research Council Member

Phytochemistry (Department of Biotechnology, New Delhi)

RC Member – CSIR, New Delhi, CDRI, Lucknow and RRL, Jammu.

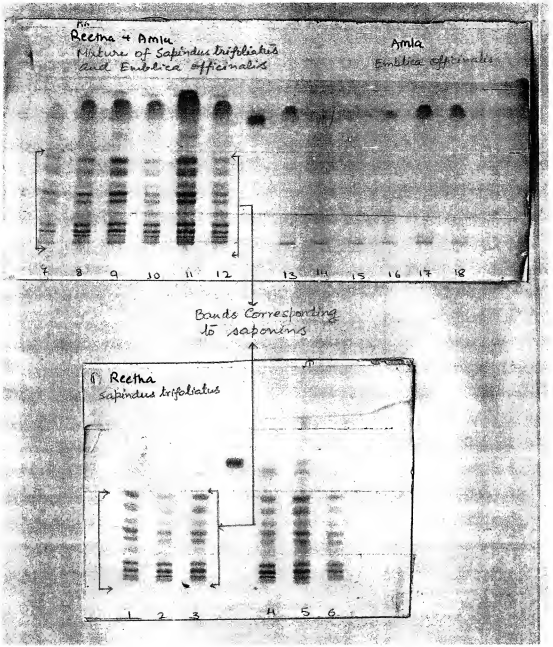
Ph.D. Guide

The following students have been awarded Ph.D. under my guidance/supervision:

1. Nawal Kishore, Ph.D. Chemistry
2. Ram Shankar Upadhayaya, Ph.D. Chemistry
3. Himadri Sen, Ph.D. Biological Science
4. Sharad Sharma, Ph.D. Biological Science
5. Rajan Goel, Ph.D. Biological Science
6. Jyoti Idnani, Ph.D. Pharmaceuticals

EXHIBIT 2

FIGURE : Comparison of Thin Layer Chromatographic (TLC) separation of the extracts and compositions of the present invention and Chikara:



Spot no. 1: Raw material of present invention (*Sapindus trifoliatus)*
Spot no. 7: (1:1) Mix of *Sapindus trifoliatus* & *Emblica officinalis* (Raw material of Chikara et. al.)
Spot no. 13: Raw material of *Emblica officinalis* as reference
Spot no. 2: Composition of the present invention (1% w/v)
Spot no. 12: Composition of Chikara (1% w/v)
Spot no. 18: Composition comprising (1%w/v) of *Emblica officinalis* as reference

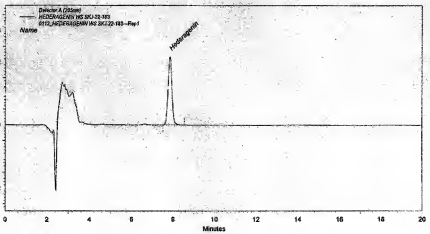
EXHIBIT 3

HPLC quantitative analysis of the compositions from Chikara and US 10/252,992 (*ref.: ANNEXURE-II*)

LUPIN LIMITED , PUNE (NCER)

Page 1 of 1

Instrument No : NCER / PK / Inst-002 Reference No : LLL2011(78) Analyst :Hemant Sugandhi
Sample Name : ***HEDERAGENIN WS SKJ-22-183***
File Name : *E:\PUBLIC\JAN-2007\LLL2011\0112_HEDERAGENIN WS SKJ-22-183---Rep1*
Method Name : E:\PUBLIC\JAN-2007\LLL2011\ASSAY Method.inct
Vial : *1* Inj. vol : *50 µL* Date Acquired : 1/13/2007 12:07:49 AM



Detector A (205nm)

Pk #	*Retention Time*	*Area Percent*	*Area*	Name
1	*7.85*	*100.00*	2844979	*Hederagenin*
Totals		*100.00*	2844979	

Figure - 1

Verified by

EXHIBIT 4

Assay report obtained from Novascreen, USA for Chikara

Cmpln etc

Assay Report

NOVASCREEN

Client Name: Lupin Ltd. (Research Park)
Client Contact: Rajan Goel
Task Order Number: 06-3662

Barcode Number: 063662-1
Client Number: LLL6738
Solubility of Stock: Soluble

Receptor	Percent Inhibition (Average; N = 2) 2.560 ug/ml	2.562 ug/ml
NEUROTRANSMITTER RELATED		
GABA A, Agonist Site	**19.21%**	**95.95%**
Glutamate, AMPA Site (Ionotropic)	-0.89%	41.69%
Glutamate, Chloride Dependant Site (Ionotropic)	**1.95%**	**84.35%**
Glutamate, Kainate Site (Ionotropic)	-1.16%	25.68%
Glutamate, NMDA Agonist Site (Ionotropic)	**15.26%**	**66.02%**
Glutamate,NMDA,Glycine (Stry-insens Site) (Ionot	4.03%	42.60%
ION CHANNELS		
GABA, Chloride, TBOB Site	-14.60%	-3.77%
Sodium, Site 2	13.37%	3.30%

Values are expressed as the percent inhibition of specific binding and represent the average of replicate tubes at each of the concentrations tested. Bolded values represent inhibition of 50% or greater.

21 January 2007

Page C-1

Assay report obtained from Novascreen, USA for Emblica officinalis alone

Assay Report

NOVASCREEN
a Caliper Life Sciences company

Client Name: Lupin Ltd.
Client Contact: Rajan Goel
Task Order Number: 06-3491

Barcode Number: 063491-1
Client Number: LLL-3064
Solubility of Stock: Soluble

Receptor	Percent Inhibition (Average; N = 2) 2.5E0 ug/ml	2.5E2 ug/ml
NEUROTRANSMITTER RELATED		
GABA A, Agonist Site	**64.09%**	**102.22%**
Glutamate, AMPA Site (Ionotropic)	**-10.07%**	**56.20%**
Glutamate, Chloride Dependent Site (Ionotropic)	28.28%	49.24%
Glutamate, Kainate Site (Ionotropic)	-1.12%	39.30%
Glutamate, NMDA Agonist Site (Ionotropic)	**14.92%**	**71.82%**
Glutamate,NMDA,Glycine (Stry-insens Site) (Ionot	0.48%	28.21%
ION CHANNELS		
GABA, Chloride, TBOB Site	-1.95%	-0.03%
Sodium, Site 2	7.11%	3.93%

Values are expressed as the percent inhibition of specific binding and represent the average of replicate tubes at each of the concentrations tested. Bolded values represent inhibition of 50% or greater.

EXHIBIT 5

NOVASCREEN, USA uses a criteria of 50% inhibition or greater to qualify a compound as active in binding experiments

NOVASCREEN

Interpreting your Data

Screening assays can provide valuable information about a compound's biological activity and selectivity. To understand and assess your data, NOVASCREEN suggests these guidelines for interpretation of the data presented:

Baseline, -20% to +20% inhibition:
In most assays, our standard baseline range runs from -20% to +20% inhibition of binding or enzyme activity. NOVASCREEN considers compounds showing results in this range inactive at this site.

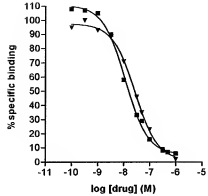
Compounds which show negative inhibition(< 20%):
NOVASCREEN's assays are designed to test for inhibition of binding or enzyme activity. Occasionally, compounds, particularly naturally derived products and extracts, will demonstrate high negative inhibition (i.e., resulting from the extraction procedure used) and may, at the discretion of the client, warrant retesting at lower concentrations.

Compounds which show inhibition in the range of 20% to 49%:
Compounds exhibiting these results show marginal activity at the receptor site and generally do not warrant further examination unless otherwise directed by the client.

Compounds which show inhibition of 50% and greater:
NOVASCREEN uses a criteria of 50% inhibition (or greater) to qualify a compound as active. Active compounds tested at multiple concentrations can generally be expected to show a dose-dependent response and such follow-up studies are recommended at the client's discretion.

EXHIBIT 6

GLUTAMATE, AMPA SITE BINDING ASSAY



Reference Compounds	Ki (nM)
■ Quisqualic Acid	15.8
▼ AMPA HBr	22.3
L-Glutamate	190.0
CNQX	299.0
NMDA	10,000
Kainic Acid	33,000

Assay Characteristics:

K_D (binding affinity):	28.0 nM
B_{max} (receptor number):	71 fmol/mg protein

Materials and Methods:

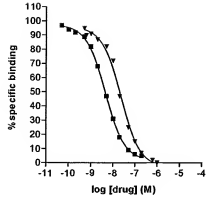
Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]AMPA (40-70 Ci/mmol) Final concentration - [5.0 nM]
Non-specific Determinant:	(+/-) AMPA - [100 µM]
Reference Compound:	(+/-) AMPA
Positive Control:	(+/-) AMPA
Incubation Conditions:	Reactions are carried out in 10 mM K_2HPO_4/100 mM KSCN (pH 7.5) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto filters is determined and compared to control values in order to ascertain any interactions of test compound with the AMPA binding site.

Literature Reference:

Murphy, et al. Characterization of Quisqualate Recognition Sites in Rat Brain Tissue Using [^{3}H]Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic Acid and a Filtration Assay. *Neurochem. Res.* **12:** 775-781 (1987) with modifications.

Morgan, R.C., et al. Binding of [^{3}H]AMPA to Non Chaotrope, Non Detergent Treated Rat Synaptic Membranes: Characteristics and Lack of Effect of Barbiturates. *Neurochem. Int.* **18(1):** 75-84 (1991).

$GABA_A$, AGONIST SITE BINDING ASSAY



Reference Compounds	Ki (nM)
■ Muscimol	4.4
Isoguvacine	9.5
▼ GABA	23.1
THIP	25.1

Assay Characteristics:

K_D (binding affinity):	370 nM
B_{max} (receptor number):	0.7 pmol/mg protein

Materials and Methods:

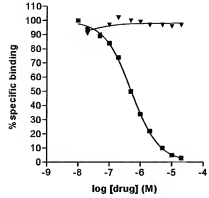
Receptor Source:	Bovine cerebellar membranes
Radioligand:	[^{3}H]GABA (70-90 Ci/mmol) Final ligand concentration - [5.0 nM]
Non-specific Determinant:	GABA - [1.0 uM]
Reference Compound:	GABA
Positive Control:	GABA
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the $GABA_A$ receptor.

Literature Reference:

Enna, S., et al. Stereospecificity and Structure-Activity Requirements of GABA Receptor Binding in Rat Brain. *Brain Research.* **124: 185-190 (1977)** with modifications.

Falch, E., Hedegaard, A., et al. Comparative Stereostructure - Activity Studies on $GABA_A$ and $GABA_B$ Receptor Sites and GABA Uptake using Rat Brain Membrane Preparations. *Jrnl. Neurochem.* **47(3): 898-903 (1986).**

GLUTAMATE, CHLORIDE DEPENDENT SITE BINDING ASSAY



Reference Compounds	Ki (nM)
■ L-Glutamate	761
▼ NMDA	>10,000

Assay Characteristics:

K_D (binding affinity):	1,134 nM
B_{max} (receptor number):	25.6 fmol/mg tissue (wet weight)

Materials and Methods:

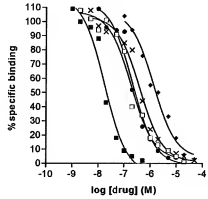
Receptor Source:	Rat cerebellar membranes
Radioligand:	[^{3}H]Glutamate (40-80 Ci/mmol) Final ligand concentration - [200 nM]
Non-specific Determinant:	L-Glutamic acid - [100 uM]
Reference Compound:	L-Glutamic acid
Positive Control:	L-Glutamic acid
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 37^{0}C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glutamate binding site.

Literature Reference:

Slevin, J., Collins, J., Lindsley, K. and Coyle, J. T. Specific Binding of [^{3}H]-L-Glutamate to Cerebellar Membranes: Evidence for Recognition Site Heterogeneity. *Brain Research.* **249:** 353-360 (1982) with modifications.

Cha, J-H. J., Makowiec, R. L., Penney, J. B., and Young, A. B. L-[^{3}H]Glutamate Labels the Metabotropic Excitatory Amino Acid Receptor in Rodent Brain. *Neurosci. Letters.* **113:** 78-83 (1990).

GLUTAMATE, NMDA, GLYCINE (STRYCHNINE-INSENSITIVE) SITE BINDING ASSAY



Reference Compounds	Ki (nM)
■ MDL 105,519	17.1
□ Glycine	181.3
● 5,7-DCKA	203.1
× D-Serine	389.7
♦ HA 966	1281.0

Assay Characteristics:

K_D (binding affinity):	20 nM
B_{max} (receptor number):	29 pmol/mg tissue (wet weight)

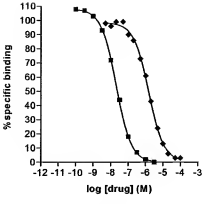
Materials and Methods:

Receptor Source:	Rat cortical (with hippocampus) membranes
Radioligand:	[^{3}H]MDL-105,519 (50-90 Ci/mmol) Final ligand concentration - [4.0 nM]
Non-specific Determinant:	MDL-105,519 - [3.0 μM]
Reference Compound:	MDL-105,519
Positive Control:	MDL-105,519
Incubation Conditions:	Reactions are carried out in 50 mM Tris-Acetate (pH 7.4) at room temperature for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glycine binding site.

Literature Reference:

Baron, *et.al.*. Pharmacological characterization of MDL-105,519, an NMDA receptor glycine site antagonist. *European Journal of Pharmacology*. **323**: 181-192 (1997).

GLUTAMATE, KAINATE SITE
BINDING ASSAY



Reference Compounds	Ki (nM)
■ Kainic Acid	9.2
L-Glutamate	220.0
◆ Kainic Acid Dimethyl Ester	914.0
NMDA	>200,000
AMPA	>200,000

Assay Characteristics:

K_d (binding affinity):	16.0 nM
B_{max} (receptor number):	400 fmol/mg protein

Materials and Methods:

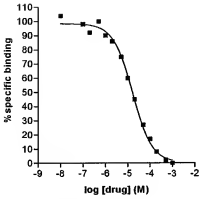
Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Kainic acid (30-60 Ci/mmol) Final ligand concentration - [10.0 nM]
Non-specific Determinant:	Kainic acid - [10 uM]
Reference Compound:	Kainic acid
Positive Control:	Kainic acid
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl buffer (pH 7.1) at 2°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the kainic acid binding site.

Literature Reference:

London, E. and Coyle, J. Specific Binding of [^{3}H]Kainic Acid to Receptor Sites in Rat Brain. *Mol. Pharmacol.* **15:** 492-505 (1979) with modifications.

Hall, R.A., Kessler, M., and Lynch, G. Kainate Binding to the AMPA Receptor in Rat Brain. *Neurochemical Research.* **19(6):** 777-782 (1994).

GLUTAMATE, NMDA AGONIST SITE
BINDING ASSAY



Reference Compounds	Ki (nM)
■ NMDA	9,300

Assay Characteristics:

K_D (binding affinity):	7.0 nM
B_{max} (receptor number):	0.77 pmol/mg tissue

Materials and Methods:

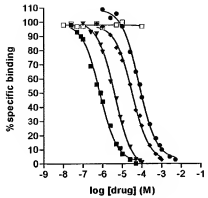
Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]CGP 39653 (25-60 Ci/mmol) Final ligand concentration - [2.0 nM]
Non-specific Determinant:	NMDA - [300 uM]
Reference Compound:	NMDA
Positive Control:	NMDA
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-Acetate (pH 7.4) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filter is determined and compared to control values in order to ascertain any interactions of test compound with the NMDA binding sites.

Literature Reference:

Lehmann, J., Hutchinson, A.J., et al. CGS 19755, A Selective and Competitive N-Methyl-D-Aspartate Type Excitatory Amino Acid Receptor Antagonist. *Jrnl. Pharmac. Exp. Ther.* **246:** 65-75 (1988) with modifications.

Murphy, D.E., Schneider, J., et al. Binding of [^{3}H]-3-(2-Carboxypiperazin-4-yl)Propyl-1-Phosphonic Acid to Rat Brain Membranes: A Selective, High Affinity Ligand for N-Methyl-D-Aspartate Receptors. *Jrnl. Pharmac. Exp. Ther.* **240:** 778-784 (1987) with modifications.

SODIUM CHANNEL, SITE 2
BINDING ASSAY



Reference Compounds	Ki (μM)
Dibucaine	0.9
■ Aconitine	1.0
Tetracaine	2.8
▼ Veratridine	3.6
Bupivacaine	3.8
◆ Lidocaine	29.9
● Procaine	92.3
Procainamide	189.0
□ Tetrodotoxin	>100.0

Assay Characteristics:

K_D (binding affinity):	32 nM
B_{max} (receptor number):	52 fmol/mg tissue (wat weight)

Materials and Methods:

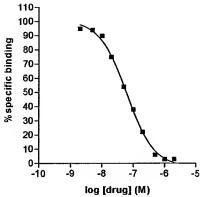
Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Batrachotoxin (30-60 Ci/mmol) Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Aconitine - [1.0 mM]
Reference Compound:	Aconitine
Positive Control:	Aconitine
Incubation Conditions:	Reactions are carried out in 50 mM HEPES (pH 7.4) containing 130 mM choline chloride at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sodium channel, site 2 binding site.

Literature Reference:

Creveling, C. R. Batrachotoxin-induced Depolarization and [^{3}H]Batrachotoxin - A 20a - Benzoate Binding in a Vesicular Preparation from Guinea Pig Cerebral Cortex. *Mol. Pharmacol.* 23: 350-358 (1983) with modifications.

Trainer, V.L., Moreau, E., et al. Neurotoxin Binding and Allosteric Modulation at Receptor Sites 2 and 5 on Purified and Reconstituted Rat Brain Sodium Channels. *Jnl. Biol. Chem.* 268(23): 17114-17119 (1993).

$GABA_A$ CHLORIDE CHANNEL, TBOB SITE BINDING ASSAY



Reference Compounds	Ki (nM)
■ TBPS	48.3
Diltiazem	>10,000
Saxitoxin	>10,000
Charybdotoxin	>10,000

Assay Characteristics:

K_D (binding affinity): 45 nM
B_{max} (receptor number): 116.7 fmol/mg tissue (wet weight)

Materials and Methods:

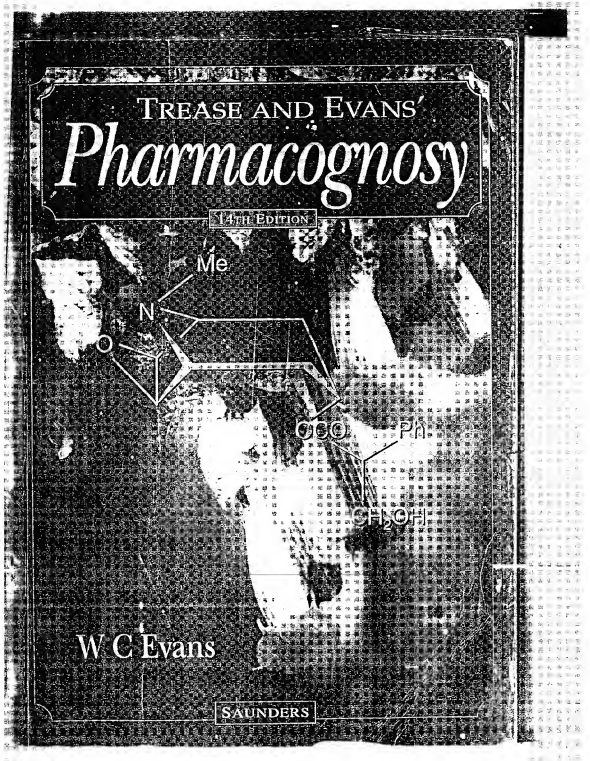
Receptor Source: Rat cortical membranes
Radioligand: [^{3}H]TBOB (20-60 Ci/mmol)
Final ligand concentration - [20 nM]
Non-specific Determinant: T-butylbicyclophosphorothionate (TBPS) - [10 µM]
Reference Compound: T-butylbicyclophosphorothionate (TBPS)
Positive Control: T-butylbicyclophosphorothionate (TBPS)
Incubation Conditions: Reactions are carried out in 20 mM $NaKPO_4$/500 mM NaCl (pH 7.5) at 25°C for 75 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the TBOB binding site.

Literature Reference:

Lawrence, L., Palmer, C., Gee, K., Wang, Yamamura, H. and Casida, J. T[^{3}H]butylbicycloorthobenzoate: A New Radioligand Probe for the Gamma-Aminobutyric Acid-Regulated Chloride Ionophore. *Jrnl. Neurochem.* **45(3):** 798-804 (1986) with modifications.

Cole, L.M., Lawrence, L.J., and Casida, J.E. Similar Properties of [^{35}S]t-butylbicyclophosphorothionate Receptor and Coupled Components of the GABA Receptor-Ionophore Complex in Brains of Human, Cow, Rat, Chicken, and Fish. *Life Sci.* **35:** 1755-1762 (1984).

EXHIBIT 7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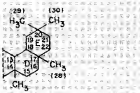
22 Saponins, Cardioactive Drugs and Other Steroids

Plant materials containing saponins have long been used in many parts of the world for their detergent properties. For example, in Europe the root of *Saponaria officinalis* (Caryophyllaceae) and in South America the bark of *Quillaia saponaria* (Rosaceae). Such plants contain a high percentage of glycosides known as saponins (Latin *sapo*, soap) which are characterized by their property of producing a frothing aqueous solution. They also have haemolytic properties, and when injected into the blood stream, are highly toxic. The fact that a plant contains haemolytic substances is not proof that it contains saponins, and in the species examined by Wall (1961) only about half of those containing haemolytic substances actually contained saponins. When taken by mouth, saponins are comparatively harmless. Sarsaparilla, for example, is rich in saponins but is widely used in the preparation of nonalcoholic beverages.

Steroid skeleton



Pentacyclic triterpenoid skeleton

Saponins have a high molecular weight and a high polarity and their isolation in a state of purity presents some difficulties. Often they occur as complex mixtures with the components differing only slightly from one another in the nature of the sugars present, or in the structure of the aglycone. Various chromatographic techniques have been employed for their isolation. As glycosides they are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids. According to the structure of the aglycone or sapogenin, two kinds of saponin are recognized—the steroidal (commonly tetracyclic triterpenoids) and the pentacyclic triterpenoid types (see formulae below). Both of these have a glycosidal linkage at C-3 and have a common biogenetic origin via mevalonic acid and isoprenoid units.

A distinct subgroup of the steroidal saponins is that of the steroidal alkaloids which characterize many members of the Solanaceae. They possess a heterocyclic nitrogen-containing ring, giving the compounds basic properties (as an example see solasodine, Fig. 22.5).

STEROIDAL SAPONINS

The steroidal saponins are less widely distributed in nature than the pentacyclic triterpenoid type. Phytochemical surveys have shown their presence in many monocotyledonous families, particularly the Dioscoreaceae (e.g. *Dioscorea* spp.), Amaryllidaceae (e.g. *Agave* spp.) and Liliaceae (e.g. *Yucca* and *Trillium* spp.). In the dicotyledons the occurrence of diosgenin in fenugreek (Leguminosae) and of steroidal alkaloids in *Solanum* (Solanaceae) is of potential importance. Some species of *Strophanthus* and *Digitalis* contain both steroidal saponins and cardiac glycosides (q.v.). Examples of saponins and their constituent sugars are given in Table 22.1.

Steroidal saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. Some are used as starting

Table 22.1. Examples of steroidal saponins.

Steroidal saponin	*Sugar components*	*Occurrence*
Sarsaponin (Parillin)	3 glucose, 1 rhamnose	*Smilax* spp.
Digitonin	2 glucose, 2 galactose, 1 xylose	Seeds of *Digitalis purpurea* and *D. lanata*
Gitonin	1 glucose, 2 galactose, 1 xylose	Seeds and leaves of *D. purpurea* and seeds of *D. lanata*
Dioscin	1 glucose, 2 rhamnose	*Dioscorea* spp.

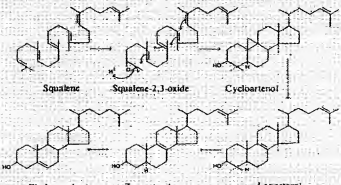


Fig. 22.1. Possible route for the formation of cholesterol in higher plants and algae.

materials for the synthesis of these compounds. Diosgenin is the principal sapogenin used by industry but most yams, from which it is isolated, contain a mixture of sapogenins in the glycosidic form.

As with cardiac glycosides, the stereochemistry of the molecule is of some importance, although not so much so for cortisone manufacture. Natural sapogenins differ only in their configuration at carbon atoms 3, 5 and 25, and in the spirostane series the orientation at C-22 need not be specified (cf. steroidal alkaloids). Mixtures of the C-25 epimers—for example, diosgenin (Δ^5,25α-spirosten-3β-ol) and yamogenin (Δ^5,25β-spirosten-3β-ol)—are of normal occurrence and their ratio, one to the other, is dependent upon factors such as morphological part and stage of development of the plant. In some instances in the plant, the side-chain which forms ring F of the sapogenin is kept open by glycoside formation as in the bisdesmosidic saponin sarsaparilloside of *Smilax aristolochiaefolia*.

Biogenesis of steroidal saponins

Steroidal saponins arise via the mevalonic acid pathway; the preliminary stages have been discussed in Chapter 16. A scheme for the subsequent cyclization of squalene to give cholesterol is illustrated in Fig. 22.1. Cholesterol, the wide distribution of which in plants has only relatively recently been shown, can be incorporated into a number of C_{27} sapogenins without side-chain cleavage (Fig. 22.2), although it is not necessarily an obligatory precursor. Extensive investigations involving whole plants, homogenates and cell cultures have been performed to elucidate these detailed pathways, including the origin of the 25-epimers (e.g. diosgenin and yamogenin).

As early as 1947 Marker and Lopez had postulated that steroidal saponins exist in plants in a form where the side-chain is held open by glycoside formation. However, direct evidence for the natural occurrence of these compounds was not forthcoming for another 20 years. It has been shown that such open-chain saponins are, like the more common ones, formed from cholesterol. In *Dioscorea* homogenates one such compound has been converted to dioscin (a diosgenin glycoside) (Fig. 22.3).

Natural steroids for the production of pharmaceuticals

Although *total* synthesis of some medicinal steroids is employed commercially, there is also a great demand for natural products which will serve as starting materials for their *partial* synthesis.

As indicated in Fig. 22.4, which illustrates the range of steroids required medicinally, cortisone and its derivatives are 11-oxosteroids, whereas the sex hormones, including the oral contraceptives, and the diuretic steroids have no oxygen substitution in the C-ring. Fig. 22.5 shows some of the more important natural derivatives which are available in sufficient quantity for synthetic purposes. Hecogenin with C-ring substitution provides a practical starting material for the synthesis of the corticosteroids, whereas diosgenin is suitable for the manufacture of oral contraceptives and the sex hormones. Diosgenin, however, can also be used for corticosteroid synthesis by the employment, at a suitable stage in the synthesis, of a microbiological fermentation to introduce oxygen into the 11α-position of the pregnene nucleus.

Efforts are constantly being made to discover new high-yielding strains of plants and to assure a regular supply of raw material by the cultivation of good-quality plants. Hardman in a review on steroids (*Planta Med.*, 1987, 53, 233) records that, annually, the American *Chemical Abstracts* contain some 3000 references perti-